Platform: Protein Conformation

123-Plat
Structure Refinement of Protein Low Resolution Models using GNEIMO Constrained Dynamics Method
In-Hee Park[1], Vamshi Gangupomu[1], Jeffrey Wagner[1], Abhinandan Jain[2], Nagarajan Vaidehi[1].
[1]Beckman Research Institute of the City of Hope, Duarte, CA, USA, [2]Jet Propulsion Laboratory, California Institute of Technology, Pasadena, CA, USA.
The challenge in protein structure prediction using homology modeling is the lack of reliable methods to refine the low resolution homology models. All-atom molecular dynamics (MD) does not serve well for structure refinement due to its limited conformational search. We have developed and tested the constrained MD method, based on the Generalized Newton-Euler Inverse Mass Operator (GNEIMO) algorithm for protein structure refinement. In this method the high frequency degrees of freedom are placed as hard holonomic constraints and a protein is modeled as a collection of rigid body clusters connected by flexible hinges that are torsions. This allows larger integration time step and enhances the conformational search space. In this work, we have demonstrated the use of GNEIMO method for protein structure refinement using no constraints, starting from low-resolution decoy set derived from homology methods. In the eight proteins with three decoys for each, we observed an improvement of ~2 Å in the RMSD to the known experimental structures of these proteins. The GNEIMO method also showed enrichment in the population density of native-like conformations. In addition, we demonstrated structural refinement using the ''Freeze and Thaw'' clustering scheme in the GNEIMO framework as a viable tool for enhancing localized conformational search. We have derived a robust protocol, based on the GNEIMO replica exchange method, for protein structure refinement that can be readily extended for other proteins and possibly used for high throughput protein structure refinement.

124-Plat
Computing Entropies for Binding and Refinement of Protein Structures
Michael T. Zimmermann, Robert L. Jernigan.
Iowa State University, Ames, IA, USA.
Entropy plays an important role in protein folding and binding, but is difficult to evaluate; consequently its contribution to free energy is often neglected or locally approximated. We propose a novel method for calculating the change in free energy upon binding using coarse-grained models of proteins by combining multi-body potentials with entropies calculated from elastic network models (ENMs). Multi-body energy potentials have been developed to account for the cooperativity in globular protein structures by an improved accounting of three dimensional interactions and residue packing. From ENM, mean square fluctuations (MSF) can be calculated using collective contributions from the entire structure. MSF are related to entropy because they represent the conformational states accessible in the native ensemble. Energies evaluated from coarse-grained multi-body potentials combined with MSF-based entropies improves selection of CASP9 targets, where each target has hundreds of decoys. Because the method shows improvement in decoy selection, we test its ability to identify native-like poses in protein-protein docking, showing positive initial results. ClusPro was used to dock pairs of structures, resulting in 11-30 candidate poses for each pair. Our combined method chooses more native-like poses in nearly all cases. If some constraints about the residues involved in binding is provided to ClusPro, the most native-like pose is often identified. For a more rigorous test, we generate 2000 poses for select structure pairs using Zdock again showing significantly better discrimination compared to results using energy alone. Accounting for the entropic contributions from the entire structure represents an important step forward in protein structure evaluation.

125-Plat
ANM Normal Modes Show the Directions for Protein Structure Refinement
Pawel Gniewek[1], Andrzej Kolinski[2], Robert L. Jernigan[3], **Andrzej Kloczkowski**[1].
[1]The Ohio State University, Columbus, OH, USA, [2]University of Warsaw, Warsaw, Poland, [3]Iowa State University, Ames, IA, USA.
It is well recognized that thermal motions of atoms in the protein native state, which correspond to fluctuations about the minimum of the global free energy, are well reproduced by simple Elastic Network Models (ENMs) such as Anisotropic Network Model (ANM). Elastic Network Models are based on the assumption that all nodes of the network (usually represented, according to the level of coarse graining of the model, by all heavy atoms or by C-alpha atoms only) that are sufficiently close together, based on a predefined cutoff distance, are connected by harmonic or more sophisticated springs. These models provide reliable representation of the fluctuational dynamics of proteins, and indicate the range of various conformational changes in protein structure, e.g. upon ligand binding. the present paper we analyze the problem of protein structure refinement by using thermal motions of proteins in nonnative states. We represent the conformational space close to the native state by a set of decoys generated by the I-TASSER protein structure prediction server utilizing template-free modeling. The protein sub-states were represented by decoys selected from the sets generated by I-TASSER by structure clustering. Based on such selected representatives some dynamical features of nonnative protein states were analyzed in detail and compared. The main finding is that for some of such substates space spanned by their thermal motions overlap significantly with deformation needed to obtain the native state. These findings are used to evaluate their possible applications for protein tertiary structure prediction from amino acid sequence and for structure refinement of low resolution models.

126-Plat
Structure-Based Design of Circularly Permutated and Frame-Shifted Photoactive Yellow Proteins
Andrew Woolley, Anil Kumar, Sameer Al-Abdul-Wahid.
University of Toronto, Toronto, ON, Canada.
Photoswitchable proteins are powerful tools for external manipulation and probing of complex biochemical processes. In an effort to create new families of photoswitchable proteins that undergo novel types of conformational changes, we designed a circularly permutated PYP (c-PYP) and frame-shifted PYP (fs-c-PYP) variants of photoactive yellow protein (wt-PYP), a relatively small blue-light sensitive protein from Halorhodospira halophila that contains a photoactive 4-hydroxycinnamic acid chromophore. The c-PYP was created by connecting the N- and C-terminal of wt-PYP with a defined linker polypeptides and introducing new N- and C-termini at G115 and S114 respectively. A fs-c-PYP was created by duplication of a beta-sheet segment in c-PYP so that it can form an alternate H-bond registry with an existing beta-sheet. The designed proteins c-PYP and fs-c-PYP are highly soluble and well folded when overexpressed in E. coli, each undergoes PYP-like photocycle upon exposure to blue light. The thermal relaxation of the protein to the dark-adapted state after removal of the blue light source varies considerably between the designed constructs. UV-Vis absorption data indicate that fs-c-PYP recovers much more slowly than wt-PYP whereas c-PYP recovers more quickly than wt-PYP. Fluorescence-monitored GdnMHCl denaturation experiments show fs-c-PYP is the least stable and wt-PYP the most stable dark-state structure, while folding/unfolding of both c-PYP and fs-c-PYP is more cooperative than wt-PYP. This work shows that new classes of photo-controlled conformational change can be created by altering the backbone topology of known photoswitchable proteins.

127-Plat
Design Strategy for High Free-Energy States of Proteins Based on High-Pressure NMR Study: Alternatively Folded Conformation of Ubiquitin
Ryo Kitahara[1], Soichiro Kitazawa[1], Maho Yagi-Utsumi[2,3], Akemi Ido[4], Makoto Urushitani[4], Kenji Sugase[5], Koichi Kato[2,3].
[1]Ritsumeikan University, Kusatsu, Shiga, Japan, [2]Okazaki Institute for Integrative Bioscience, Okazaki, Aichi, Japan, [3]Nagoya City University, Nagoya, Aichi, Japan, [4]Shiga University of Medical Science, Otsu, Shiga, Japan, [5]Suntory Foundation for Life Sciences, Osaka, Japan.
We demonstrate a rational design for the high Gibbs-free energy conformer N2 of ubiquitin based on the solution structure of the N2 state of the wild-type protein, previously characterized by high pressure NMR spectroscopy. Using a single amino acid replacement method, we created the ubiquitin mutant that would favor the population of N2 conformer. Structure, dynamics and stability of the N2 state are characterized by 1H, 15N, 13C chemical shifts, 15N-spin relaxation (1H 600-950 MHz), water-amide proton exchange (CLEANEX-PM) and residual dipolar coupling experiments. All the NMR investigations indicate that the ubiquitin mutant represents an open conformer suitable for enzyme recognition similar to that observed for the N2 state of the wild type protein, namely swinging out of the a-helix region with a simultaneous reorientation of the C-terminal segment. Examinations of functional activities for the mutant protein indicate that the mutant retains abilities of the polyubiquitination through the E1-E2-E3 cascade reactions and association with the ubiquitin interacting motif UIM. Investigation of the mutant protein of ubiquitin, which is the first model of a stable high-energy state of the protein N2, is useful for further structural and functional studies of ubiquitin and ubiquitin-related systems.